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PRINCIPAL INVESTIGATORS: Milton F. Bryant, M.D.
Staff, Piedmont Hospital

Walter L. Bloom M.D.
Director of Medical Education
And Research
Piedmont Hospital

Spencer S. Brewer, M.D.
Staff, Piedmont Hospital

INSTITUTION: The Ferst Research Laboratory
Piedmont Hospital
Atlanta, Georgia

STUDY OF THE ANTI-THROMBOTIC PROPERTIES OF DEXTRANS
OF VARIOUS MOLECULAR WEIGHTS

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3. Principal Investigators: Milton F. Bryant, M.D.
Staff, Piedmont Hospital
Walter L. Bloom, M.D.
Director of Medical Education
And Research
Piedmont Hospital
Spencer S. Brewer, M.D.
Staff, Piedmont Hospital
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1. Experimental Study of the Anti-Thrombotic Properties of Dextrans Of Low Molecular Weight. A standardized preparation in the dog has been used to evaluate the effect of dextrans of low molecular weight in preventing thrombosis in small arteries subjected to surgical trauma. In 23 preparations given an infusion of dextran, with an average molecular weight of 43,000, the two hour thrombotic rate was 50 percent. The thrombotic rate in 12 preparations treated with NRC-2B dextran, average molecular weight 34,000, was 90 percent and in ten preparations treated with NRC-2, average molecular weight 11,800, the thrombotic rate was 75 percent. It would appear that dextrans of low molecular weights are not as effective as clinical dextran in preventing intravascular thrombosis.

2. Study of the Anti-Thrombotic Properties of Dextrans of Large Molecular Weight. The anti-thrombotic properties of dextrans with high average molecular weight have been evaluated using a standardized experimental preparation that has a known post-traumatic thrombotic rate of 95 percent. In 93 preparations treated with dextrans of large average molecular weights, the thrombotic rate was reduced to 59 percent. These studies suggest that clinical dextran is superior to dextrans of higher molecular weights in preventing thrombosis in small arteries of dogs subjected to a standardized surgical trauma.

3. Coating of Vascular Surfaces and Cells - A New Concept in the Prevention of Intravascular Thrombosis. Radiocarbon (C-14) labeled

dextran has been used to study the mechanism of action of dextran. It is to be emphasized that the studies reported are preliminary but they definitely indicate that dextran coats all the intravascular surfaces. The ability of dextran to coat surfaces may be its fundamental mechanism of action in retarding intravascular thrombosis.

EXPERIMENTAL STUDY OF THE ANTI-THROMBOTIC
PROPERTIES OF DEXTRANS OF LOW MOLECULAR
WEIGHT

Milton F. Bryant, M.D.

Walter L. Bloom, M.D.

Spencer S. Brewer, M.D.

With the technical assistance of:

Jeanne Clark, MT ASCP

Carol Ann Gray, R.N.

The Ferst Research Laboratory

Piedmont Hospital

Atlanta, Georgia

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EXPERIMENTAL STUDY OF THE ANTI-THROMBOTIC PROPERTIES OF
DEXTRANS OF LOW-MOLECULAR WEIGHT

Dextran has been used extensively as a plasma volume expander.
A number of reports^{1,2,3,4} have shown that infusion of large amounts of macromolecular substances, such as acacia, polyvinyl alcohol, gelatin, and dextran, may prolong the bleeding time. The hemostatic defect associated with the intravenous administration of more than 1000 ml. of clinical dextran has not been clearly defined; however, the reported defects are probably related to changes in several of the systems involved in clot formation. Recent studies by Bloom⁵ and associates suggest that prolongation of bleeding time is more closely associated with expansion of whole blood volume than to plasma dextran concentration.⁶ Stefanini and Dameshek report changes in fibrinogen conversion and a decrease in total platelet counts following clinical dextran infusions, and Behrmann and Hartman⁷ describe hypofibrinogenemia, thrombocytopenia and low plasma fibrinogen after plasma volume expansion with dextran.⁸ Other workers report no deleterious effects upon the coagulation mechanism following infusion of so-called "low-molecular weight dextran". The changes in hemostasis produced by clinical dextran suggested that this glucosidic polysaccharide might prevent or retard intravascular thrombus formation. The use of clinical dextran in preventing thrombosis in small arteries which have been traumatized has been previously reported.⁹ This report is a continuation of our studies evaluating the effect

of dextrans of low molecular weight in preventing thrombosis in small arteries subjected to a standardized mechanical trauma.

MATERIAL AND METHODS

These studies were performed in dogs using the standard preparation previously described.⁹ Briefly, the femoral artery was exposed and a two centimeter segment measuring 2.5 - 3 mm. in external diameter was isolated. A longitudinal arteriotomy was performed and the intima was carefully stripped from this segment. The arteriotomy incision was closed with # 7-0 arterial silk. Prior to releasing the vascular clamps an infusion of dextran was given and a catheter was inserted into the urinary bladder for collection of urine samples.

In 23 preparations an infusion of dextran* with an average molecular weight of 43,000 was given in quantities equal to one percent of body weight. Ten preparations were given a one percent infusion of NRC-2B, average molecular weight of 34,000; and in 12 preparations a one percent infusion of NRC-2, average molecular weight of 11,800, was given. After intimestomy and dextran infusion the vascular clamps were removed and a pulsatile flow of blood was restored to the traumatized segment. The preparation was observed continuously for two hours and the time required for an occluding thrombus to form in the intimestomized segment was determined by inspection and palpation, and then recorded. Previous studies revealed that 90 percent of 40 similar untreated control preparations thrombosed within

* Supplied by Baxter Laboratories.

the two hour observation period.

Blood and urine samples were collected at intervals for 24 hours and in certain instances for eight days. The concentration of dextran in the plasma and urine was determined by the method described¹⁰ by Bloom and Wilcox.

Blood samples were also obtained for thrombelastographic analysis and for measurement of the thrombin and prothrombin times. In selected instances sedimentation rates were performed prior to and following the dextran infusion.

RESULTS

In 23 intimestomized preparations given an intravenous infusion of "dextran 43,000", thrombosis occurred within the two hour observation period in 12 instances giving a thrombotic rate of 52 percent, Fig. 1. All of the preparations functioned well initially. The onset of clot formation was rapid and always began at the distal end of the suture line. The clot gradually progressed proximally to involve the entire traumatized segment. As the obstructing thrombus formed the vessel gradually lost its elastic properties and began to pulsate in a piston-like fashion longitudinally.

Plasma dextran levels revealed an average concentration of 575 mg. percent one hour following the infusion. The blood concentration fell rapidly with a concomitant rise in urinary excretion. Approximately 50 percent of the dextran was excreted in six hours and in 48 hours the plasma dextran level had returned to normal.

The thrombelastographic changes are similar to those reported for clinical dextran, Fig. 2. This pattern was obtained one hour follow-

ing an infusion of "dextran 43,000". The major change in the pattern is a decrease in ma value from 56 to 40. The r value is not significantly affected; however the k value is increased. "Dextran 43,000" produced an average increase in the prothrombin time of 1.6 seconds and an average decrease in the fibrindex time of 1.2 seconds. The average prothrombin time prior to the infusion was 17.2 seconds and the average time one hour following the infusion was 18.8 seconds. The average control sedimentation rate was 9mm/hour and dextran with an average molecular weight of 43,000 produced little or no change in this rate. All of these coagulation studies returned to normal as the dextran was cleared from the blood stream.

In ten preparations a one percent infusion of NRC-2B was given prior to releasing the vascular clamps. Thrombosis occurred in nine of these preparations giving a thrombotic rate of 90 percent Fig.3.

Plasma and urine samples revealed a rapid excretion of NRC-2B. The average one hour blood level was 370 mg. percent, and in six hours it had fallen to 75 mg. percent. The urine concentration was inversely related to the plasma level and both urine and blood dextran levels were essentially normal in 24 hours. NRC-2B was found to decrease the sedimentation rate.

The thrombelastographic pattern obtained one hour following infusion of NRC-2B showed a definite decrease in ma value, Fig. 4. No change in the r or k values was noted. The average prothrombin time was increased from 17.2 seconds to 18.7 seconds and the average fibrindex time was decreased from 5.6 seconds to 5.3 seconds.

In 12 preparations a one percent infusion of NRC-2 was given. Thrombosis occurred in eight instances giving a thrombotic rate of 75 percent, Fig. 5.

Blood and urine concentration studies revealed a very rapid excretion of NRC-2. The plasma and urine dextran levels returned to normal within two to six hours.

The thrombelastographic changes produced by NRC-2, Fig. 6, were similar to, but not as marked as, the changes described for NRC-2B. NRC-2 caused a decrease in the average prothrombin time from 17.2 seconds to 16.4 seconds and a decrease in the average fibrin index time from 5.6 seconds to 4.9 seconds. As with NRC-2B, the sedimentation rate was found to be decreased by a one percent infusion of NRC-2 dextran, Fig. 7. Changes in the measured coagulating mechanism returned to normal as the plasma dextran level returned to normal.

DISCUSSION

9

In a previous study it was found that the two hour thrombotic rate in 40 untreated intimestomized preparations was 90 percent. These preparations must be carefully prepared as described in order that mechanical factors will not influence the thrombotic rate. Meticulous removal of all shredded tissue from the traumatic segment is essential. It is important to place the sutures so as to prevent constriction of the artery. We have not found the dissecting microscope to be necessary in suturing these preparations. Another prerequisite for a satisfactory preparation is that it must transmit a pulsatile flow of blood for ten minutes or more after the

vascular clamps are removed.

The thrombotic rate following infusion of "dextran 43,000" was 52 percent as compared to a thrombotic rate of ten percent following infusions of clinical dextran. NRC-2B and NRC-2 dextran had no significant influence on the thrombotic rate. It would appear that dextrans of low molecular weight are not as effective as clinical dextran in preventing clot formation in small arteries subjected to a standardized surgical trauma. The total plasma dextran levels were equally elevated with all weights of dextran during the two hour observation period. This finding suggests that the anti-thrombotic properties of dextran are related to molecular size rather than total plasma dextran concentration.

It is known that dextrans of low molecular weight increase the suspension stability of red cells.¹¹ This action of "dextran 43,000" (frequently called low-molecular weight dextran) has been suggested^{12,13} by a number of workers as being helpful in preventing blood sludging and the many pathologic states which have been ascribed to it. Recent studies by Pories and associates¹⁴ cast doubt upon the harmful effects of erythrocyte aggregation. In the present investigation clinical dextran increased the sedimentation rate and at the same time markedly reduced the thrombotic rate. NRC-2 and NRC-2B dextran have very low molecular weights and decrease the sedimentation rate; however, in the quantities used it did not influence the development of thrombosis in the experimental preparations. Since dextran is a non-polar polysaccharide one would expect various molecular weights of this substance to increase or decrease the sedimentation

rate in accordance with known physical chemical laws. The relationship between suspension stability and clot formation is, as yet, not clearly defined.

As expected, dextrans of small molecular weights are rapidly excreted by the kidneys. NRC-2 dextran, average molecular weight 11,800, appears in the urine in large quantities immediately following intravenous administration. Within a period of six hours this substance was cleared from the plasma. NRC-2B and "dextran 43,000" have larger average molecular weights and are less rapidly cleared from the plasma. A significant amount of clinical dextran, average molecular weight 75,000, remains in the plasma for five days.

Previous reports have shown that "dextran 43,000" has little or no effect on the clotting mechanisms as studied. Thrombelastography is a valuable means of photokymographically recording the effect of dextran upon the kinetics of clot formation. As with clinical dextran it was found that dextrans of low molecular weights primarily affected the ma value. NRC-2 and NRC-2B did not have as pronounced an effect on the ma value as did "dextran 43,000". The magnitude of change in the thrombelastographic pattern may be partially dependant on the presence of a definite amount of dextran of a particular molecular size, and more detailed studies will be reported elsewhere.

SUMMARY

A standardized preparation in the dog has been used to evaluate the effect of dextrans of low molecular weights in preventing thrombosis in small arteries subjected to surgical trauma. In 23 preparations given an infusion of dextran, with an average molecular

weight of 43 000. the two hour thrombotic rate was 50 percent. The thrombotic rate in 12 preparations treated with NRC-2B dextran, average molecular weight 34,000 was 90 percent and in ten preparations treated with NRC-2, average molecular weight 11,800, the thrombotic rate was 75 percent. When these thrombotic rates are compared with a thrombotic rate of ten percent following clinical dextran infusion, it would appear that dextrans of low molecular weights are not as effective as clinical dextran in preventing intravascular thrombosis.

REFERENCES

1. Heckel, C.P., Erickson, C.C., Wuille, C.L., Smith, R.E.: Blood Plasma Proteins as Influenced by Intravenous Injection of Gum Acacia. J. Exp. Med. 67:345, 1938.
2. Hueper, W.C., Landsberg, J.W., Eskridge, L.C.: The Effects of Intravenous and Intraperitoneal Introduction of Polyvinyl Alcohol Solutions Upon the Blood. J. Pharm. and Exp. Therapy. 70: 201, 1940.
3. Horvath, S.M., Hamilton L.H., Spurr, G.B., Allbaugh, E.B., Hutt, B.K.: Plasma Expanders and Bleeding Times. J. Applied Phys. 7:614, 1955.
4. Carbone, J.V., Furth, R.W., Scott, R.Jr., Crosby, W.H.: An Hemostatic Defect Associated with Dextran Infusion. Proc. Soc. Exp. Biol. and Med. 55:101, 1954.
5. Bloom, W.L., Fowler, N.O., Ward J.A., Flanch, R.H.: Unpublished Data.
6. Stefanini, M., Dameshek, W.: Hemorrhagic Disorders. Grune and Stratton, New York, 1955.
7. Behrmann, V.G., Hartman, F.W.: Studies on Capillary Resistance After Intravenous Infusions. Fed. Proc. 13:10, 1954.
8. Long, D.M., Sanchez, L., Varco R.L., Lillehei, C.W.: The Use of Low Molecular Weight Dextran and Serum Albumin as Plasma Expanders in Extracorporeal Circulation. Surgery, 50:12-28, 1961.

Continuation of References:

9. Bryant, M.F., Bloom, W.L., Brewer, S.S.: Use of Dextran in Preventing Thrombosis of Small Arteries Following Surgical Trauma, J.M.A.G., 50, No. 12 Dec. 1961.
10. Bloom W.L., Wilcox, M.L.: Determination of Dextran in Blood and Urine, Proc. Soc. Exp. Biol. and Med. 76:3, 1951.
11. Swank, R.I.: Suspension Stability of the Blood After Injection of Dextran, J. Appl. Physiol., 12:125, 1958.
12. Gelin, L.B., Zederfeldt B.: Low Molecular Weight Dextran--A Rheologic Agent Counteracting Capillary Stagnation, Acta. Chir. Scand. 119:168, 169, 1960.
13. Thorsen, G., Hint, H.: Aggregation, Sedimentation and Intravascular Sludging of Erythrocytes, Acta. Chir. Scand. Suppl. 154, 1950.
14. Pories, W.J., Harris, P.D., Hinshaw, J.R., Davis, T.P., Schwartz, S.I.: Blood Sludging: An Experimental Critique of its Occurrence and its Supposed Effects, Ann. Surg. 155:33-41, 1962.

DEXTRAN
(43,000)

TOTAL INTIMECTOMIES	23
THROMBOSIS IN 2 HOURS	12
THROMBOTIC RATE	52%

Fig. 1. Thrombotic Rate Following Dextran (43,000).

DEXTRAN
(43,000)

THROMBELASTOGRAPHIC PATTERN
FOLLOWING DEXTRAN (43,000)

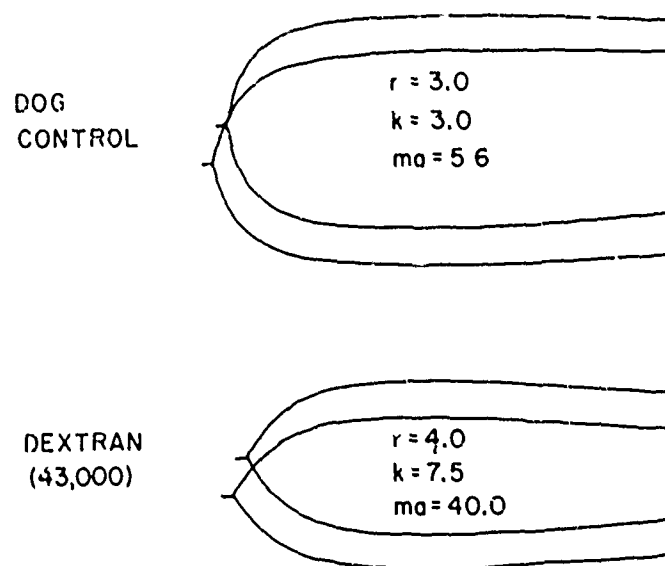


Fig. 2. Thrombelastographic Pattern
Following Dextran (43,000)

DEXTRAN
(34,000)

TOTAL INTIMECTOMIES	10
THROMBOSIS IN 2 HOURS	9
THROMBOTIC RATE	90%

Fig. 3. Thrombotic Rate Following
Dextran NRC-2B (34,000).

DEXTRAN

(34,000)

THROMBELASTOGRAPHIC PATTERN
FOLLOWING NRC-2B (34,000)

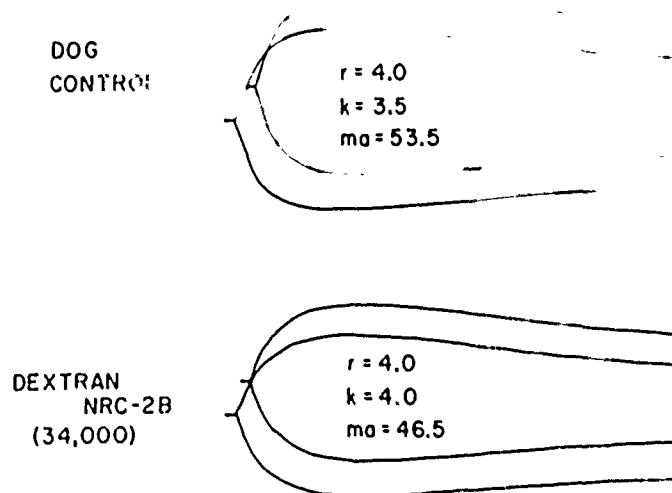


Fig. 4. Thrombelastographic Pattern
Following Dextran NRC- 2 B
(34,000)

DEXTRAN
(11,800)

TOTAL INTIMECTOMIES	12
THROMBOSIS IN 2 HOURS	8
THROMBOTIC RATE	75 %

Fig. 5. Thrombotic Rate Following Dextran
NRC-2 (11,800).

DEXTRAN

(11,800)

THROMBELASTOGRAPHIC PATTERN
FOLLOWING NRC-2 (11,800)

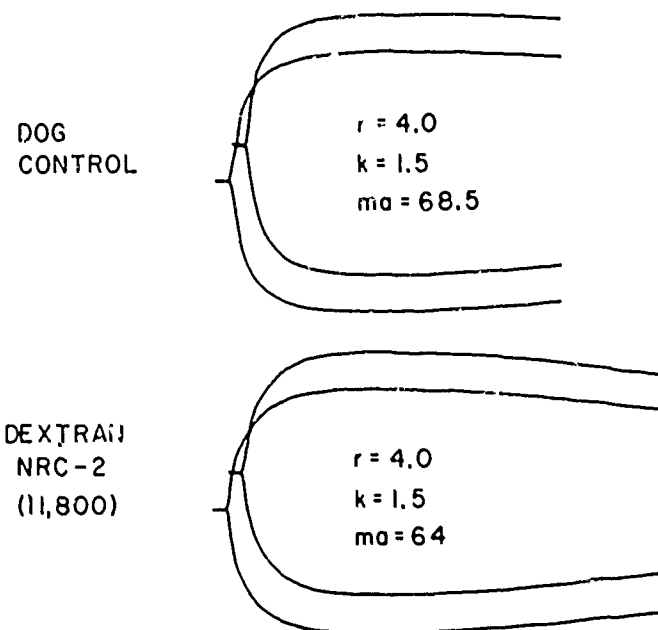


Fig. 6. Thrombelastographic Pattern

Following Dextran NRC-2 (11,800)

SEDIMENTATION RATE
(WINTROBE)

CLINICAL DEXTRAN	-	INCREASED
DEXTRAN (43,000)	-	NO CHANGE
NRC-2B (34,000)	-	DECREASED
NRC-2 (11,800)	-	DECREASED

Fig. 7. Comparison Of Effect On Sedimentation
Rate.

STUDY OF THE ANTI-THROMBOTIC PROPERTIES
OF DEXTRANS OF LARGE MOLECULAR WEIGHT

Milton F. Bryant, M.D.

Walter L. Bloom, M.D.

Spencer S. Brewer, M.D.

With the technical assistance of:

Jeanne Clark, MT ASCP

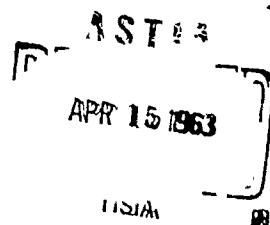
Carol Ann Gray, R.N.

The Ferst Research Laboratory

Piedmont Hospital

Atlanta, Georgia

Supported by U.S. Army Contract No. DA-49-193-MD-2168



STUDY OF THE ANTI-THROMBOTIC PROPERTIES
OF DEXTRANS OF LARGE MOLECULAR WEIGHT

A continued search for an effective method of preventing post-operative thrombosis in small arteries has been carried out in this laboratory during the past two years. ^{1,2,3,4,5.} Heparin and two fibrinolytic enzyme preparations were found to be only partially effective and impractical to use. Our studies have been reported indicating that an infusion of clinical dextran (6% solution of MW 75,000) in quantities equal to one percent of the body weight is responsible for the prevention of post-operative thrombosis in small ¹ arteries. Dextran of low molecular weight are not as effective as clinical dextran in preventing thrombosis in arteries following ³ a standardized mechanical trauma. Since dextrans of MW 75,000 and less are quantitatively removed from the intravascular compartment in a matter of a few hours it was felt that study of the anti-thrombotic properties of dextrans with average molecular weights larger than the albumin molecule might add to our understanding of the protective effect observed.

MATERIAL AND METHODS

The materials and methods used in this study were the same as reported previously. The femoral arteries of mongrel dogs weighing between 15 and 20 pounds were exposed so as to be able to isolate a two centimeter segment that measured 2.5 to 3 mm. in external diameter. Bulldog clamps were applied and a longitudinal arteri-

otomy was made in the 2 cm. segment. A # 15 knife blade was used to mechanically scrape all of the intima from the isolated segment. The arteriotomy incision was closed with # 7-0 arterial silk in a continuous fashion. The bulldog clamps were then released and a pulsatile flow of blood was restored through the traumatized segment. The vessel was kept moist with saline and continuously observed for two hours or until thrombosis of the segment occurred. The time for an occluding thrombus to form was recorded in each study. Thrombosis was determined by palpation and inspection and in selected instances by continuously recording the pressure distal to the traumatized segment. Since the thrombotic rate in a large series of control experimental preparations was known, this study was not repeated, but random control animals were interposed in the study to verify reproductability of method and thrombosis.

In order to determine the effect of dextrans of large average molecular weights on intravascular thrombosis, a group of dogs was prepared as described in the control animals. In each animal a six percent solution of dextran was infused in volume to equal one percent of the animal's weight.

GROUP ONE - Thirty-five intimestomy preparations in 10 dogs were treated with dextran of an average molecular weight of 185,000.

GROUP TWO- Twenty intimestomy preparations in ten dogs were treated with dextran of an average molecular weight of 300,000.

GROUP THREE - Twenty intimestomy preparations in ten dogs were treated with dextran of an average molecular weight of 450,000.

GROUP FOUR - Eighteen intimestomy preparations in nine dogs were treated with dextran of an average molecular weight of 500,000.

GROUP FIVE - In order to determine the isolated effect of blood volume expansion on the thrombotic rate of the standardized experimental preparation, a one percent infusion of Polyvinylpyrrolidone was used to treat 14 intimestomized arterial segments in seven dogs. A Thrombotest assay (Owren), a thrombin time (fibrinogen assay), thrombelastographic studies, and phase contrast platelet counts were performed on representative dogs infused with dextrans of various molecular weights. Comparative platelet counts were performed in two dogs receiving dextran 500,000 and in three dogs receiving dextran 185,000. Blood and urine dextran levels were determined along with the coagulation studies in selected instances.

RESULTS

In group one, thirty-five intimestomized arteries in dogs were treated with dextran of an average molecular weight of 185,000. Thrombosis of the traumatized segment occurred in 20 instances after restoration of a pulsatile blood flow. A thrombotic rate

<u>Number of thrombosis</u>	of 57 percent resulted (Fig.1)
Number of intimestomies	

Dextran 300,000 was used in group two. Thrombosis occurred in 11 instances resulting in a thrombotic rate of 55 percent.

Twelve of 20 experimental preparations in group three thrombosed following a one percent infusion of dextran 450,000 providing a thrombotic rate of 60 percent.

In group four, dextran with an average molecular weight of 500,000

was used to treat 18 intimestomized preparations and an occluding thrombus developed in 12 instances. The thrombotic rate for this group was 66 percent.

A total of 93 surgical preparations were treated with dextrans of high average molecular weight. Thrombosis occurred within the two hour observation period in 55 instances giving a thrombotic rate of 59 percent. (Fig.2).

Polyvinylpyrrolidone was used to treat 14 preparations in group five and in each instance thrombosis of the intimestomized segment occurred within the two hour observation period. The thrombotic rate was 100 percent and of the same order of magnitude found in the control series.

These dextran preparations in the quantities used did not produce any significant change in the Thrombotest assay (Owren); the average control prothrombin time was 17.8 seconds and the average time one hour following the infusion was 18.4 seconds (Fig.3). The thrombin time was slightly but consistently shortened by a one percent infusion of the various dextrans studied. Prior to the dextran infusion the average thrombin time was 6.1 seconds and one hour following the dextran infusion, the average time was 4.7 seconds.

The thrombelastograph pattern was altered consistently by all of the dextran preparations studied. A marked decrease in the ma value occurred routinely following an infusion of large molecular weight dextran. In addition, the r and k values increased. As dextran leaves the intravascular compartment the thrombelastographic pattern returns to normal. These results will be reported in detail elsewhere.

Platelet Counts - Platelet counts were done on the "platelet-rich" plasma samples, an aliquot of which was used for the thrombelastographic measurement. Phase contrast microscopy was used to insure the greatest possible accuracy in the direct method counting since the morphology of dog platelets allows for easy confusion with other methods. Numerically, platelets are not altered significantly by dextran infusions. Functionally, they appear to be markedly altered, not in their contribution to the formation of an active thromboplastic complex, but in their contribution to clot strength. Thus, "dextran coated platelets" are rendered "thrombasthenic".

Blood and urine dextran determinations confirm the fact that these large dextran molecules remain in the intravascular compartment, and usually at a high concentration, for a much longer time than clinical dextran. The time of dextran residence in the intravascular compartment was directly related to the average molecular weight of the dextran preparation infused (Fig.4). Following a single intravenous infusion of dextran 500,000 (the largest preparation studied) the blood dextran levels were found to remain elevated for six days.

DISCUSSION

Previous study revealed that the two hour thrombotic rate in the untreated intimestomy preparations was 95 percent. It was also shown that a one percent infusion of clinical dextran would reduce the thrombotic rate to ten percent. Numerous reports on the beneficial effects of low molecular weight dextran in preventing sludging and

thrombus formation in the micro-circulation stimulated an evaluation of the anti-thrombotic properties of dextrans of low average molecular weights.^{6,7,8.} Using for comparison the thrombotic rate in the standard intimestomy preparations, dextrans of low average molecular weights were found to be less effective than clinical dextran in preventing post-traumatic thrombosis.³

Since dextrans of large average molecular weights remain in the intravascular compartment longer than clinical dextran, it was anticipated that these dextrans might possess greater anti-thrombotic properties than clinical dextran. It was also felt that these large molecules would reside in the vascular bed for a longer duration of action. The intimestomy preparation in the dog revealed that the large dextran molecules do possess some effect in preventing post-traumatic thrombosis but the effect is not as great as found with clinical dextran.

The phenomenon of intravascular aggregation or sludging and the prevention of this phenomenon by low molecular weight dextran has been studied extensively by many investigators.^{6,7,8,9.} Rheomacrodex is of pharmacologic importance primarily because of its ability to prevent or reverse aggregation of blood corpuscles. Dextrans of high molecular weights have been shown to increase the erythrocyte sedimentation rate and cause red cell aggregation and stasis as determined by microscopic examination of the vital circulation. By producing sludging one might expect large dextran molecules to increase the thrombotic rate in the experimental preparation. Actually, these large molecules demonstrated an anti-thrombotic effect under

the conditions of this study. The explanation for the results observed in this and other studies must await a more precise definition of the mechanism of action of the dextran molecule.

10,11.

Bloom and others have shown that one of the important physiological actions of dextran is related to the increase in colloidal osmotic pressure and resulting plasma volume expansion. Polyvinylpyrrolidone was infused to expand the plasma volume in 14 experimental preparations and an occluding thrombus developed in all of the preparations. The amount of PVP infused resulted in an increase in plasma volume that was equal to the volume expansion produced by a one percent infusion of clinical dextran. Assuming that PVP does not have some unknown effect that initiates thrombus formation it would appear that volume expansion alone does not affect the thrombotic rate in the standard experimental preparations used in this study.

Dextrans of high molecular weights did not produce any significant change in the Thrombotest (Owren). These substances produced (as did clinical and low molecular weight dextrans) a shortening of the thrombin time. An infusion of dextran of any molecular weight in a six percent concentration consistently produces a decrease in the r value of the thrombelastographic pattern. All of the large molecular weight preparations produced an increase in the r value whereas MW 75,000 and smaller preparations produced a shortening of the r value. These findings suggest that large molecules of dextran may interfere with those protein moieties involved in the initiation of clot formation.

SUMMARY

The anti-thrombotic properties of dextrans with high average molecular weights have been evaluated using a standardized experimental preparation that has a known post-traumatic thrombotic rate of 95 percent. In 93 preparations treated with dextrans of large average molecular weights, the thrombotic rate was reduced to 59 percent. This compares with a thrombotic rate of only ten percent in preparations treated with clinical dextran. These studies suggest that clinical dextran is superior to dextrans of higher molecular weights in preventing thrombosis in small arteries of dogs subjected to a standardized surgical trauma.

DEXTRAN (HMWD) TREATED PREPARATIONS

<u>Weight</u>	<u>No. Treated</u>	<u>No. Thrombosed</u>	<u>Thrombotic Rate</u>
Dextran 185,000	35	20	57%
Dextran 300,000	20	11	55%
Dextran 450,000	20	12	60%
Dextran 500,000	18	12	66%

Fig. 1. Rate of Thrombosis Following High Molecular Weight Dextran.

HIGH MOLECULAR WEIGHT DEXTRAN TREATED PREPARATIONS

Total Intimectomies - 93

Thrombosis in 2 Hours - 55

Thrombosis Rate - 59%

Fig. 2. Average Thrombotic Rate Following HMW Dextran.

COAGULATION STUDIES

	Prothrombin Time	Fibrindex	MA	r	k	Platelet Count
Dextran 185,000	N.C.	↓	↓	↑	↑	sl.↓
Dextran 300,000	N.C.	↓	↓	↑	↑	
Dextran 450 000	N.C.	↓	↓	↑	↑	
Dextran 500,000	N.C.	↓	↓	↑	↑	sl.↓

Fig. 3. Summary of Coagulation Studies Following HMW Dextran

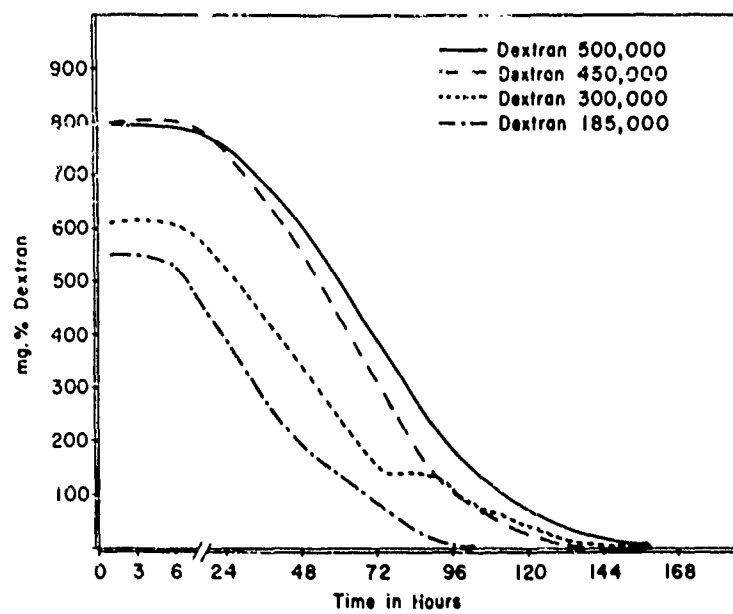


Fig. 4 COMPARISON OF BLOOD DEXTRAN LEVELS

REFERENCES

1. Bryant, M.F., Brewer, S.S., Bloom, W.L.: Use of Dextran in Preventing Thrombosis in Small Arteries Following Surgical Trauma, J. of the Med. Assoc. Georgia, Dec: 580-583, 1961.
2. Bryant, M.F., Brewer, S.S., Bloom, W.L.: Use of Dextran in Preventing Thrombosis of Small Arteries, Clinical Research, Vol.10, No. 1, P. 49, Jan. 1962, Abstract.
3. Bryant, M.F., Bloom, W.L., Brewer, S.S.: Experimental Study of the Anti-thrombotic Properties of Dextrans of Low Molecular Weight, The Amer. Surgeon, (To be published).
4. Bryant, M.F., Bloom, W.L., Brewer, S.S.: A New Method of Preventing Post-Operative Thrombosis in Small Arteries, J. of the Southern Med. Assoc., (to be published).
5. Bloom, W.L., Harmer, D., Bryant, M.F., Brewer, S.S.: Coating of Blood Vessel Surfaces and Blood Cells - A New Concept in the Prevention of Intravascular Thrombosis, Circulation, Part 2, Oct. 1962.
6. Gelin, L.B., Zederfeldt, B.: Low Molecular Weight Dextran - A Pheologic Agent Counteracting Capillary Stagnation, Acta Chir. Scand. 119:168-169, 1960.
7. Long, D.M., Sanchez, L., Varco, R.L., Lillehei, C.W.: The Use of Low Molecular Weight Dextran and Serum Albumin as Plasma Expanders in Extracorporeal Circulation, Surgery, 50:12-28, 1961.
8. Gelin, L.B., Zederfeldt, B.: Low Viscous Dextran as a Therapeutic Against Capillary Stagnation, European Conference on

References continued:

- Microcirculation, Hamburg, 1960.
9. Thorsen, G., Hint, H.: Aggregation, Sedimentation and Intravascular Sludging of Erythrocytes, Acta. Chir., Scand. Suppl. 154, 1950.
 10. Bloom, W.L.: Present Status of Plasma Expanders in the Treatment of Shock, Archives of Surgery, 63:739-741, 1951.
 11. Fowler, N.O., Franch, R.H., Bloom, W.L.: Hemodynamic Effects of Anemia Without Plasma Volume Expansion, Circulation Research, 4:319, 1956.

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COATING OF VASCULAR SURFACES AND CELLS - A NEW CONCEPT
IN THE PREVENTION OF INTRAVASCULAR THROMBOSIS

W.L. Bloom, M.D.

D.S. Harmer, PhD.

M.F. Bryant M.D.

S.S. Brewer, M.D.

With the technical assistance of:

Jeanne Clark, MT ASCP

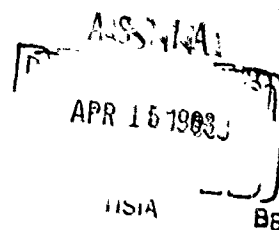
Carol Ann Gray, R.N.

The Ferst Research Laboratory

Piedmont Hospital

Atlanta, Georgia

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Intravascular thrombosis is a major cause of morbidity and mortality in our present society. Arteries and veins are frequently occluded by clots which produce varying degrees of organ damage and patient disability. At the present time methods for treating intravascular thrombosis include direct surgical removal of the clot, dissolution of the clot with enzymes, and prevention of new clot formation or extension by altering the concentration of the various factors involved in blood coagulation. Although these methods provide aids for patient care, obvious inadequacies remain in our treatment of intravascular thrombosis. It appears that intravascular clot formation results from a concentration of critical reactants at a focus of injury in the blood vessel. Attempts to alter the reactants involved in coagulation have not completely solved the problem. Recently a number of investigators have challenged the effectiveness of the presently available anticoagulants in treating intravascular thrombosis. A new approach to therapy is needed.

Since the introduction of plasma volume expanders in 1918, it has been known that circulating macromolecular substances alter hemostasis to varying degrees (1-4). Although many investigators have measured different aspects of this hemostatic defect, (5-8) no totally satisfactory explanation of the changes produced by these substances on the hemostatic mechanisms has been found.

Our recent studies (9,10,11) have shown that dextran, a non-polar

polysaccharide provides a significant anti-thrombotic effect in vivo. Vascular thrombosis was reproducibly produced in 2.5 to 3 mm. arteries of dogs by subjecting the arteries to a standardized mechanical trauma. Using this experimental preparation it was shown that an infusion of clinical dextran (M.W. 75,000) in quantities equal to one percent of the animal's weight prevented thrombosis at the site of vascular damage. An overall alteration of blood coagulation in dextran treated animals was noted by thrombelastographic study (12, 13). Thrombelastographic changes were found with blood dextran concentrations which produced no other measurable alteration in blood coagulation. Thus it was found that dextran prevents thrombosis in traumatized blood vessels and produces alterations in overall coagulability. These results suggested that clinical dextran would be of help in preventing disastrous thrombosis following surgical procedures on the vascular system and a helpful substance to infuse in patients prone to the development of intravascular clots. In addition, these findings stimulated a study of the mechanisms by which dextran produces its effect on the prevention of thrombosis.

It was postulated that the observed effects of dextran on thrombosis and coagulation might be related to surface adsorption of the dextran molecule with a resultant alteration in the chemical reactions leading to coagulation or thrombosis. The determination of the mechanism of action is of prime importance, as an understanding of this mechanism might lead to the development of a more efficient method or substance to prevent or treat intravascular thrombosis.

METHOD

The purpose of this study was to test the hypothesis that the prevention of intravascular thrombosis and the alteration in hemostasis by dextran was the result of in vivo surface coating of blood vessels and formed elements. As a means of tracing the location of dextran in or on blood vessel surfaces and formed elements, radio-carbon (C-14) labeled material was used.

B-512-Dextran of molecular weight 82,000, and randomly labeled with carbon 14 was used to trace the location of the polysaccharide. The specific activity of the labeled dextran (0.36 uc/ml) corresponds to 10^{12} molecules of dextran per disintegration per minute. The counting efficiency for C-14 in dextran was measured by counting a known volume of the stock dextran which had been processed in a manner identical with the samples. The counting efficiency was found to be 20.1% so that one observed count per minute corresponds to the presence of 5×10^{12} dextran molecules. Conventional methods of counting carbon 14 were used. Radioactive dextran (1% of body weight) was injected into mongrel dogs immediately following surgical intymectomy of one femoral artery. The dextran was injected just prior to the release of the clamps occluding the vessel for intymectomy. This was the standard procedure previously reported (9 and 11), with the exception that in the current studies radioactive dextran was used.

Three experiments were performed A,B, and C. Thirty to ninety minutes after injection of dextran the animals were killed by exsanguination all blood being taken in ACD-solution to prevent

clotting. Whole blood was centrifuged for measurement of the hematocrit and for separation of cells and plasma, for later examination. The blood vessels (femoral arteries and aorta Exp. C) were frozen and sectioned with the cutting blade parallel to the intimal surface. Serial sections were floated (in water) on to the planchets, dried and counted. Photographs were taken of the dried specimens, enlargements were made and the surface area determined by planimetry.

All specimens were washed six times in 6% non-radioactive clinical dextran in saline. (This radioactive dextran is similar to the non-active dextran currently used in clinical therapy), (See Table I).

After six consecutive washings with non-radioactive dextran, the removal of all non-surface adherent material was demonstrated by absence of significant amounts of radioactivity in the last washing. Some tissues, Plasma and washed cells were digested in strong alkali (KOH in A & B and LiOH in C). After the digestion, the dextran was alcohol precipitated, washed in alcohol, plated, dried and counted. In all instances the activity was determined by gas-flow proportional counting, using a windowless counter.

RESULTS

The specifications of the radioactive dextran used in this study compare favorably with the specifications of the currently used clinical dextran. After six consecutive washings, dextran remains in or on the blood vessel and packed cells, as can be seen from Table II. It was difficult to equate quantitative measurements of the blood vessel from one animal to another in regard to activity.

however, cell mass could be measured accurately and the quantitative radioactivity in or on the cells of two different animals was similar. Since no significant radioactivity was found in the last washing of the tissue or cell preparations, it appears that the radioactive extran was adherent to the specimens studied.

The previous demonstration that dextran will prevent vascular thrombosis of the intimated femoral artery, indicated the advisability of comparison of radioactivity in damaged and undamaged blood vessel (Table III). Vessels of approximate size (Exp. B) and approximate weight (Exp. C) were studied. In both experiments significantly more radioactivity was found in the damaged blood vessel, than in the undamaged vessel from the opposite leg. At this point the experiments had shown that the radioactivity of dextran was firmly adherent in the greater amount in damaged than in undamaged blood vessels and that radioactivity was also present in the cells removed from the circulation.

It might be argued that the radioactivity was no longer in the dextran but in some other tissue substances, thus the tissues were digested and dextran precipitated. The activity was found to remain in the dextran fraction.

The next experiment was directed at establishing the location of radioactivity in the blood vessel. A diagrammatic drawing (Fig. I) shows the manner in which a six times washed aorta was sectioned while frozen. These longitudinal sections showed that radioactivity was much greater on the surface sections than on sections of the muscle layer (Table IV), where practically no activity was found.

The surface area of the plated sections from the aorta was related to the radioactivity in terms of counts per cm square per minute. Since 1 of 260 molecules are tagged and each disintegration represents 10^{12} molecules it is possible to determine the number of dextran molecules per cm square of blood vessel surface. The size of the molecule is approximately 90×3700 Angstroms and several orientations of the molecule to the surface were considered. Arrangement of the molecules with the long axis parallel to the surface would require a smaller number of molecules (3×10^{11} molecules) than were found (1.8×10^{14}). It would take 600 layers of superimposed molecules oriented in this long axis parallel manner to produce the amount of radioactivity found (Fig.2.) If the dextran molecules were arranged with the radial parallel to the surface, 1.5×10^{14} molecules would be required to saturation one square centimeter of surface, (Fig. 2). Therefore this molecular arrangement would require a number of molecules similar to the actual number of molecules found on the surface.

DISCUSSION

The data obtained in these experiments, though preliminary, show that dextran molecules adsorb on the surface of intravascular structures. Rothman, Nelson, Schwebel and Langdell (14) used radioactive dextran in vitro to demonstrate that platelets and red cells were coated with dextran. Ross and Ebert (13) confirmed platelet coating using a cell electrophoresis method. Until now the in vivo coating of both blood vessel surfaces and circulating formed elements

has been purely hypothetical (8).

The mechanism of alteration of hemostasis following dextran injection may be envisioned as steric hinderence of clotting factors at any surface by the coating of the surface with the dextran molecule. Undoubtedly, in vivo platelet coating as well as surface coating of blood vessels could prevent platelet aggregation which is envisioned as an early phase of intravascular clotting. The thrombelastographic studies are compatible with a decrease in the number of effective platelets despite relatively small decrease in platelet numbers by phase microscopy counts (11).

The coating of an area of injury could prevent the manifestation of injury from being expressed in the blood stream. This in essence would be providing an insulator between the circulating blood and the area of injury. Another mechanism of action could be that dextran combines in greater amount with the injured area by virtue a chemical bond thus neutralizing the local manifestation of injury and preventing the clotting mechanism. Previous demonstration of the clotting effect of positive charge in blood vessels (16) would fit into this latter hypothesis.

The nature of the bond between the surface and the molecule is most probably the result of a hydrogen bond. The quantitative aspects of the number of molecules per square centimeter of surface suggests that molecules are arranged with the radial axis parallel to the cell surface. This arrangement would be compatible with the terminal free aldehyde group of the dextran being involved in the surface bond.

Although the data are preliminary and will require extensive elaboration, molecular coating of intravascular surfaces by dextran has been demonstrated. Whether this coating sterically separates the clotting factors from the locus of injury, insulates the surface from the injury, or maintains the normal surface charge can be resolved with further study. Whatever the mechanism of action, surface coating by dextran molecules has been established and shown to prevent intravascular thrombosis. This new avenue of study may provide better methods and materials to prevent or treat intravascular thrombosis.

SUMMARY

Radiocarbon (C-14) labeled dextran has been used to study the mechanism of action of dextran. It is to be emphasized that the studies reported are preliminary but they definitely indicate that dextran coats all the intravascular surfaces. The ability of dextran to coat surfaces may be its fundamental mechanism of action in retarding intravascular thrombosis.

REFERENCES

1. Heckel, C.P., Erickson C.C., Yuile, C.L. and Knutti, R.E.:
Blood Plasma Proteins as Influenced by Intravenous Injection of
Gum Acacia. J. Exp. Med. 67:345, 1938.
2. Hueber, W.C., Landsberg, J.W. and Eskridge, L.C.: The Effects
of Intravenous and Intraperitoneal Introduction of Polyvinyl
Alcohol Solution Upon the Blood. J. Pharm. and Exp. Therap.
70:201, 1940.
3. Horvath, S.M., Hamilton, L.H., Spurr, G.B., Allbaugh, E.B., and
Hutt, B.K.: Plasma Expanders and Bleeding Times. J. Applied Phys.,
7:614, 1955.
4. Carbone, J.V., Furth, R.W., Scott, R., Jr. and Crosby, W.H.: An
Hemostatic Defect Associated With Dextran Infusion. Proc. Soc.
Exp. Biol. and Med., 55:101, 1954.
5. Jacobaeus Ulf.: Studies on the Effect of Dextran on the Coagu-
lation of Blood. Acta Med. Scand., 157:9-98, 1957, Suppl. 322.
6. Bergentz, S.E., Eiken, O., and Nilson, I.M.: The Effect of Dex-
tran of Various Molecular Weight on the Coagulation in Dogs.
Thrombosis et Diathesis Haemorrhagica, 6:15-24, July 15, 1961,
(Stuttgart).
7. Adelson, E., Rheingold, J.J., Parker, D., Buenaventura, A. and
Crosby, W.H.: Platelet and Fibrinogen Survival in Normal and
Abnormal States of Coagulation. Blood, 17:267-281, 1961.
8. Bloom, W.L., Fowler, N.O., Ward, J.A., Franch, R.H.: Hemo-
dilution and Changes in Hemostasis Induced by Dextran. J. Exp.
Surg. (to be published March 1963).

References Continued:

9. Bryant, M.F., Bloom, W.L., Brewer, S.S.: Use of Dextran in Preventing Thrombosis of Small Arteries Following Surgical Trauma. J. of the Med. Assoc. of Georgia, 50:580-582, 1961.
10. Bryant, M.F., Brewer, S.S. and Bloom, W.L.: Experimental Study of the Anti-Thrombotic Properties of Dextran of Low Molecular Weight. The Amer. Surgeon. (To be published).
11. Bryant, M.F., Brewer, S.S. and Bloom, W.L.: Use of Dextran in Preventing Thrombosis of Small Arteries. Abstract Clinical Research, 10:1, 1962.
12. Brewer, S.S., Bloom, W.L., Bryant, M.F.: Thrombelastographic Study of the Coagulation Defects Induced by Dextran. Clin. Research, January, 1963.
13. Brewer, S.S.: The Effect of Dextran of Various Molecular Sizes on the Coagulation Mechanism. Bull. Fulton County Med. Soc. February, 1963.
14. Rothman, S., Adelson, E., Schwebel, A. and Langdell, R.D.: Adsorption of Carbon-14 Dextran to Human Blood Platelets and Red Blood Cells, In Vitro. Vox Sanguinis, 2:104-109, 1957.
15. Ross, W.W. And Ebert, R.V.: Microelectrophoresis of Blood Platelets and the Effects of Dextran, J. Clinical Invest., 38: 155-160, 1959.
16. Sawyer, P.N. and Pate, J.W. :Bio-Electric Phenomena as an Etiologic Factor in Intravascular Thrombosis. Am J. of Phys. 175:103-107, 1953.

References Continued:

17. Sawyer, P.N., Pate, J.W. and Weldon, C.S., Relations of Abnormal and Injury Electric Potential Differences to Intravascular Thrombosis. Am. J. of Physiol., 175:108-112, 1953.

FIGURE 1

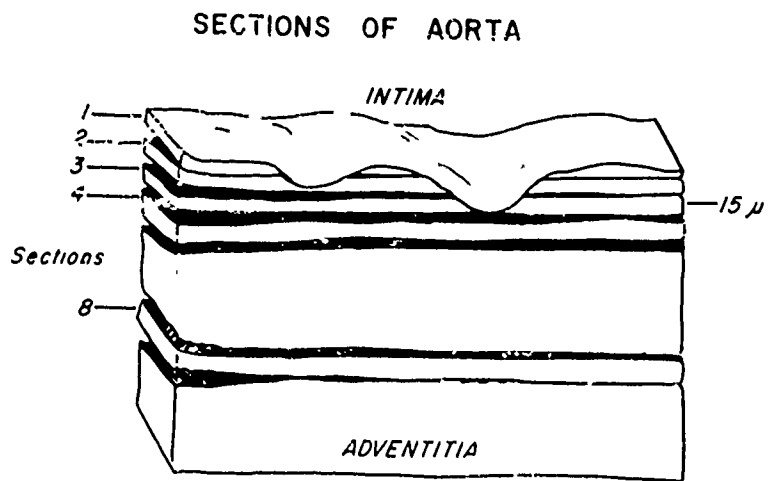


Fig. 1. Sections of Aorta.

FIGURE 2

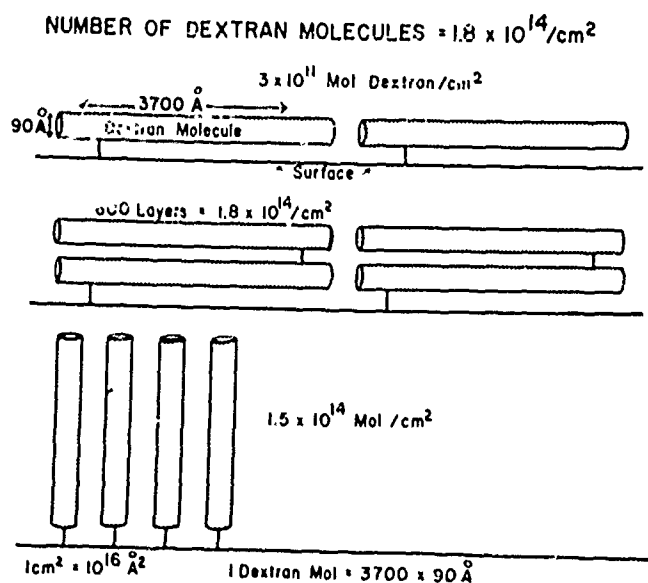


Fig. 2. Molecular Arrangement of Dextran.

TABLE I

RADIOACTIVE DEXTRAN - SPECIFICATIONS

CONCENTRATION	B 512 in Saline	6.06 gm/100 ml.
MOLECULAR WEIGHT	(Light Scattering)	82,600 AV.
INTRINSIC VISCOSITY	25° C	0.20
SPECIFIC ACTIVITY	(Combustion Carbon)	6.36 uC/gm.
MOLECULES OF DEXTRAN/DISINTEGRATION/MIN		10 ¹²
TAGGED MOLECULES		1 out of 260
SIZE	Approx. Radial Diam.	90 Angstroms
	Approx. Axial Diam.	3,700 Angstroms
COUNTING EFFICIENCY		20.1%
DOSE INJECTED		1% Body Weight

TABLE II

RADIOACTIVITY IN OR ON FORMED ELEMENTS

BLOOD VESSEL, NORMAL	A	$9. \pm 1.7$	CPM*
	B	$14.5 \pm .2$	CPM
	C	41.0 ± 1.0	CPM
1 ML. PACKED WASHED CELLS	B	$51.2 \pm .2$	CPM
	C	$50.0 \pm .2$	CPM

*Counts per minute

TABLE III

RADIOACTIVE DEXTRAN ON NORMAL AND INTINECTOMIZED

BLOOD VESSELS

	<u>Normal B.V.</u>	<u>Intinectomized B.V.</u>
Exp. B	14.5 \pm .2 CPM Approx. 1 cm ²	30.3 \pm .2 CPM Approx. 1 Cm ²
Exp. C	41.1 \pm .0 CPM Weight 49 mg.	53.4 \pm 1.0 CPM Weight 50 mg.

TABLE IV

RADIOACTIVITY ON BLOOD VESSEL SURFACE

	<u>INTIMA SECTION</u>	<u>MUSCULAR SECTION</u>
Surface Area	.098 cm ²	.512 cm ²
CPM	.35 ± .9	0 ± .09
1 Square CM	36 CPM	0 CPM